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Improving water management in European catfish recirculating aquaculture systems through catfish-lettuce aquaponics

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1710404> since 2019-08-22T13:53:06Z

Published version:

DOI:10.1016/j.scitotenv.2019.06.167

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(Article begins on next page)

1 **Improving water management in European catfish recirculating aquaculture systems through catfish-** 2 **lettuce aquaponics**

3

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14 **Abstract**

15 In the context of climate change and population growth, aquaculture plays an important role for food security,
16 employment and economic development. Intensive recirculating aquaculture systems (RAS) allow to treat
17 and recycle fish effluents to reduce waste concentration in outflow water thereby reducing environmental
18 contamination. RAS sustainability may be further improved using aquaponics, a circular productive system
19 in which RAS wastewater is recovered for crop cultivation and recycled back to the fish tanks. In this study,
20 water metabolism of a catfish RAS was assessed and the opportunity to produce lettuce with the RAS
21 effluent was tested. Crop growth and water consumption in aquaponics were compared to those experienced
22 in hydroponics at three nutrient solution concentration (EC of 1.6, 2.0 and 3.0 dS·m⁻¹), also considering water-
23 (WUE) and nitrogen- use efficiency (NUE). A scenario for converting the RAS in a catfish-lettuce aquaponic
24 system was, then, proposed.

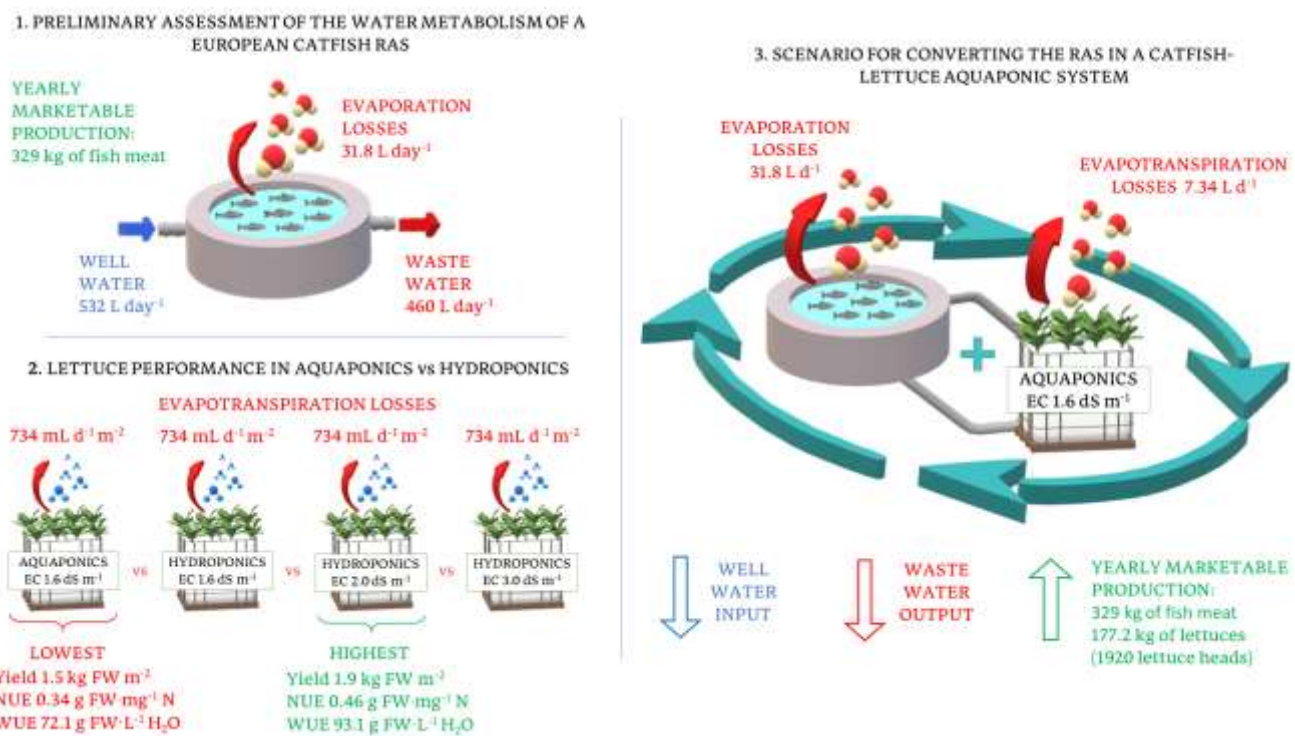
25 The RAS water balance included an input of 555 L·day⁻¹, out of which 32 L·day⁻¹ were lost by evaporation
26 from the tubs whereas 460 L·day⁻¹ were discarded. The lettuce yield, NUE and WUE in aquaponics were
27 respectively 20.3%, 22.3% and 20.6% lower than those obtained in hydroponics. Best performances in

28 hydroponics were achieved with EC of 2.0 dS m^{-1} . No difference in term of water consumption arose between
29 the treatments, with average water use of $46 \text{ mL} \cdot \text{plant}^{-1} \cdot \text{day}^{-1}$.
30 Considering the current RAS productivity of 329 kg year^{-1} , a 10 m^2 raft system hosting 160 lettuces would
31 satisfy the nitrogen filtration demand. Once closed the water loop between the two productive sub-units, the
32 current water input of $532 \text{ L} \cdot \text{day}^{-1}$ could be reduced to the amount needed to replace the water lost by
33 evaporation ($50 \text{ L} \cdot \text{day}^{-1}$) and the RAS water output would decrease from 555 to $103 \text{ L} \cdot \text{day}^{-1}$.

34

35 **Keywords: Aquaponics, RAS, electrical conductivity, water use efficiency, sustainability**

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40 **1. Introduction**

41 World population is expected to increase between 20% and 30% by 2050, growing from 7.7 billion people to

42 between 9.2 and 10.2 billion. Accordingly, global food demand is foreseen to increase by 60% by 2025

43 (Alexandros and Bruinsma, 2012). Concurrently, the global water consumption, already risen by 600% in the

44 last century, will keep growing at a 1% yearly rate Wada et al., (2016) In this framework, the FAO Fisheries

45 and Aquaculture Department (FAO, 2018) highlighted the important role of fisheries and aquaculture in

46 boosting food and nutrition security, job employment and income generation for local communities. Today

47 aquaculture (e.i. the cultivation of aquatic organisms in natural or controlled marine or freshwater

48 environments) accounts for over 50% of the fish destined for human consumption, providing a primary source

49 of protein and essential micronutrients worldwide (FAO, 2018). Current aquaculture systems range from

50 extensive to intensive, depending on the level of inputs, the fish stocking density, and the degree of

51 management. The adoption of intensive land-based systems located close to the markets, i.e. in the areas with

52 high population density, is rapidly growing. Intensive fish farming systems can be categorized in “flow-

through” and “RAS” (Recirculating Aquaculture System). The former are open-systems in which the clean water flows once and is discharged thereafter. They are common in regions with high water availability, but their use is becoming always more limited due to the stringent water regulations regarding wastewater discharges into natural water bodies. The effluents from these open systems, containing residuals of uneaten food and high concentrations of nitrogen and phosphorus, can in fact cause oxygen depletion and eutrophication in the receiving water bodies (Martins et al., 2010). In the RAS, instead, only less than 10% of the total water volume needs to be daily discarded (Timmons and Ebeling, 2017). These systems are provided of mechanical and biological filters and additional water treatment components to depurate water from pollutants and allowing diseases control. Through this filtration process, the same volume of water can be continuously recirculated within the system while only a limited amount needs to be replaced with fresh water to avoid excessive nutrient loading.

Despite the introduction of RAS in aquaculture already contributed to downsizing the wastewater emissions into the environment, the disposing of this water is still a constant concern for aquaculture operators, especially now that environmental measures are evolving toward the concept of zero emission circular economy (EU, 2018). In this context, the set-up of closed-loop productive systems with low- or no-emissions, such as the aquaponics (e.g. a soilless system for crop production integrating aquaculture and hydroponics) concept, is raising growing interest (Endut et al., 2016). A key advantage of aquaponics is the symbiotic relationship between the plants and the fishes of the horticultural and aquaculture systems, respectively, which can be connected through the recirculation of the water flow. The fish dejections in the outflow water from the aquaculture system provide nutrients for the plants growth in the horticultural one, whereas plants, in turn, clean and filter the water that can be reused back to the fish tank (Goddek et al., 2015). This mutual exchange depends on the action of two different groups of bacteria, namely *Nitrosomonas spp.* and *Nitrobacter spp.* These bacteria oxidize the ammonia and nitrites excreted by the fishes to nitrates, which are easier to absorb by the plant roots (Rakocy et al., 2006). When an appropriate balance between the fish waste generation and the plants’ nutrient uptake is achieved, the daily water consumption can be reduced to the water required for replacing the losses by evapotranspiration (Timmons and Ebeling, 2017). Accordingly, the discharge of effluents into the environment is reduced. In this sense, aquaponics promotes the minimization of by-product flows from economic activities by employing them as

resources in another activity, thereby contributing to the fulfilment of the goals of circular economy (EU, 2018). Previous researches positively evaluated the waste-to-input aquaponic technology as a sustainable approach to manage RAS wastewater, assessing the plants potential in utilizing nutrients from fishery effluents and their contribution in maintaining a proper level of water quality (Endut et al., 2016; Espinosa Moya et al., 2017). The goal of the present research is, then, to measure the water consumption of a pre-existing catfish RAS and to make a preliminary evaluation of the potential use of its wastewater for lettuce production in hydroponics, pursuant to the aquaponic principles. The specific objectives are to (a) quantify the water metabolism and losses of the existing aquaculture system, (b) compare the lettuce growth (i.e. yield, Water Use Efficiency - WUE - and Nitrogen Use Efficiency - NUE) in aquaponics vs conventional hydroponics, and (c) propose a design scenario to convert the aquaculture system into a catfish-lettuce aquaponic system. A set of experiments were therefore implemented in Soest (Germany) to address these research questions.

93

94 **2. Materials and methods**

95 **2.1. Case study**

96 Data collection was conducted at the experimental Recirculating Aquaculture System (RAS) of the South
97 Westphalia University of Applied Sciences (SWUAS) in Soest (Germany) between June 2016 and July 2017.
98 This plant was created in 2015 and consisted of three fish tanks of 1100 L each, filled up independently with
99 a water inflow processed in a sedimenter (460 L), a biofilter (nitrification process), and a water heater (800
100 L). The water outflow from the fish tanks was channeled to the sedimenter for solid removal and, then, re-
101 directed to the biofilter, thereby closing the water cycle. A certain amount of waste-water, however, was
102 discarded by the system and replaced by fresh water in order to dilute contaminant concentration and keeping
103 safe life condition both for fishes and bacteria.

104 Three different sizes of European Catfish (*Silurus glanis*) fingerlings (50, 300 and 400 g), with a stocking
105 density of 90-100 kg·m⁻³, were separately farmed in each fish tank. Feeding rates were set as a percentage of
106 the total fish biomass, starting from 4% for juveniles to 1.5% for adults. The fish feed used was commercial
107 diet pellet of 2 mm diameter for small-size fishes and 4.5 mm for large-size fishes (Aller Aqua Group,
108 Christiansfeld, Denmark) with 54% protein and 20% fat.

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2.2. Experimental setup

The experiment was carried out in two phases. First, an experimental protocol was developed to quantify the water metabolism and the water losses of the aquaculture system. Subsequently, a small scale Deep Water Culture (DWC) hydroponic system was set up in order to evaluate lettuce (*Lactuca sativa* L. cv. Salanova multileaf) yield and water consumption either using the RAS effluent or three hydroponic solutions with different nutrient concentrations.

2.2.1. RAS water balance and efficiency parameters

The daily water balance of the RAS unit was calculated (Eq. 1) including all water inputs and outputs of the water metabolism occurring in the RAS room (Fig. 1). The considered water inputs were the consumption of fresh well water (Q_{ww}) and the water vapour that entered the room through ventilation (Q_{v_in}). The water outputs were the evaporation taking place from the biofilter (Q_b), the sedimenter (Q_s), the fish tanks (Q_{ft}) and the sump tank (Q_{st}), the water assimilated by fishes (Q_f), the water vapour that escaped the room through ventilation (Q_{v_out}), and the water discarded (Q_w).

$$\text{Water inputs} = \text{Water outputs}$$

$$\text{Tap water consumption } (Q_{ww}) + \text{Ventilation } (Q_{v_in}) = \text{Evaporation } (Q_b + Q_s + Q_{ft} + Q_{st}) + \text{Fish water assimilation } (Q_f) + \text{Ventilation } (Q_{v_out}) + \text{Wastewater } (Q_w) \quad [\text{Eq. 1}]$$

Methods for defining each element of Eq. 1 are described below.

Water consumption. The amount of well water entering the system was measured daily with a water meter.

Ventilation. The water inputs and outputs as water vapour through the ventilation system of the room (expressed as $\text{kg H}_2\text{O s}^{-1}$) were calculated according to Eq. 2, based on the absolute humidity (AH, $\text{g}\cdot\text{m}^{-3}$), the air flow speed (v , $\text{m}\cdot\text{s}^{-1}$), and the area of the section crossed by the airflow (A_w , m^2).

$$Q_w \left[\frac{\text{kg H}_2\text{O}}{\text{s}} \right] = AH \left[\frac{\text{kg}}{\text{m}^3} \right] \cdot v \left[\frac{\text{m}}{\text{s}} \right] \cdot A_w [\text{m}^2] \quad [\text{Eq. 2}]$$

The absolute humidity was calculated by employing an on-line psychrometric chart (<https://www.rotronic.com>) based on air pressure, air temperature, and relative humidity of the airflow. These parameters were measured 8 times in one month and the average values were used in the on-line tool. The air flow speed was measured with a propeller anemometer.

Evaporation. The water evaporation produced in the sediment, the fish tanks and the sump tank was calculated applying the Eq. 3, based on the evaporation coefficient (θ , $\text{kg} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$), the water surface area (A_s , m^2), and the difference between the saturated air maximum humidity ratio (x_s , $\text{kg H}_2\text{O} \cdot \text{kg}^{-1}$ of dry air) and the actual air humidity ratio (x , $\text{kg H}_2\text{O} \cdot \text{kg}^{-1}$ of dry air) above the water surface.

$$Q_i \left[\frac{\text{kg H}_2\text{O}}{\text{d}} \right] = 24 \cdot \theta \left[\frac{\text{kg}}{\text{m}^2 \cdot \text{h}} \right] \cdot A_s [\text{m}^2] \cdot (x_s - x) \left[\frac{\text{kg H}_2\text{O}}{\text{kg dry air}} \right] \quad [\text{Eq. 3}]$$

The evaporation coefficient, θ , results from Eq. 4, where v is the velocity of the air above the water surface ($\text{m} \cdot \text{s}^{-1}$).

$$\theta \left[\frac{\text{kg}}{\text{m}^2 \cdot \text{h}} \right] = 25 + 19v \left[\frac{\text{m}}{\text{s}} \right] \quad [\text{Eq. 4}]$$

Note that the units of θ do not match with each other as it is a purely empirical formula.

The actual air humidity ratio (x) can be expressed as the ratio between the partial pressure of vapor in moist air (p_w) to the atmospheric pressure of moist air (p_a), where the factor 0.622 is the ratio between the molecular weight of water vapour ($18.015 \text{ g} \cdot \text{mol}^{-1}$) and the average molecular weight of the other atmospheric gases ($28.965 \text{ g} \cdot \text{mol}^{-1}$) (Shi et al., 2017) (Eq. 5).

$$x = \frac{0.662 p_w [\text{Pa}]}{p_a [\text{Pa}] - p_w [\text{Pa}]} \quad [\text{Eq. 5}]$$

164 The actual air humidity ratio and the saturated air humidity ratio were calculated based on the air relative
 165 humidity, the air temperature, and the atmospheric pressure, using two online calculators of the thermodynamic
 166 properties of moist air (<https://www.rotronic.com> and <http://www.tlv.com>), according to Hyland and Wexler
 167 (1983). The relative humidity and the temperature of the air above the water surfaces of the fish tanks, the
 168 sedimenter and the sump were measured with three different sensors, recording data each ten seconds. The air
 169 speed at the water surface level was assumed as $0.05 \text{ m}\cdot\text{s}^{-1}$ (Smith et al., 2014).
 170 The water evaporation from the biofilter was calculated considering the difference between the biofilter
 171 ventilation outflow humidity when the biofilter is switched on and when it is off (Eq. 6).

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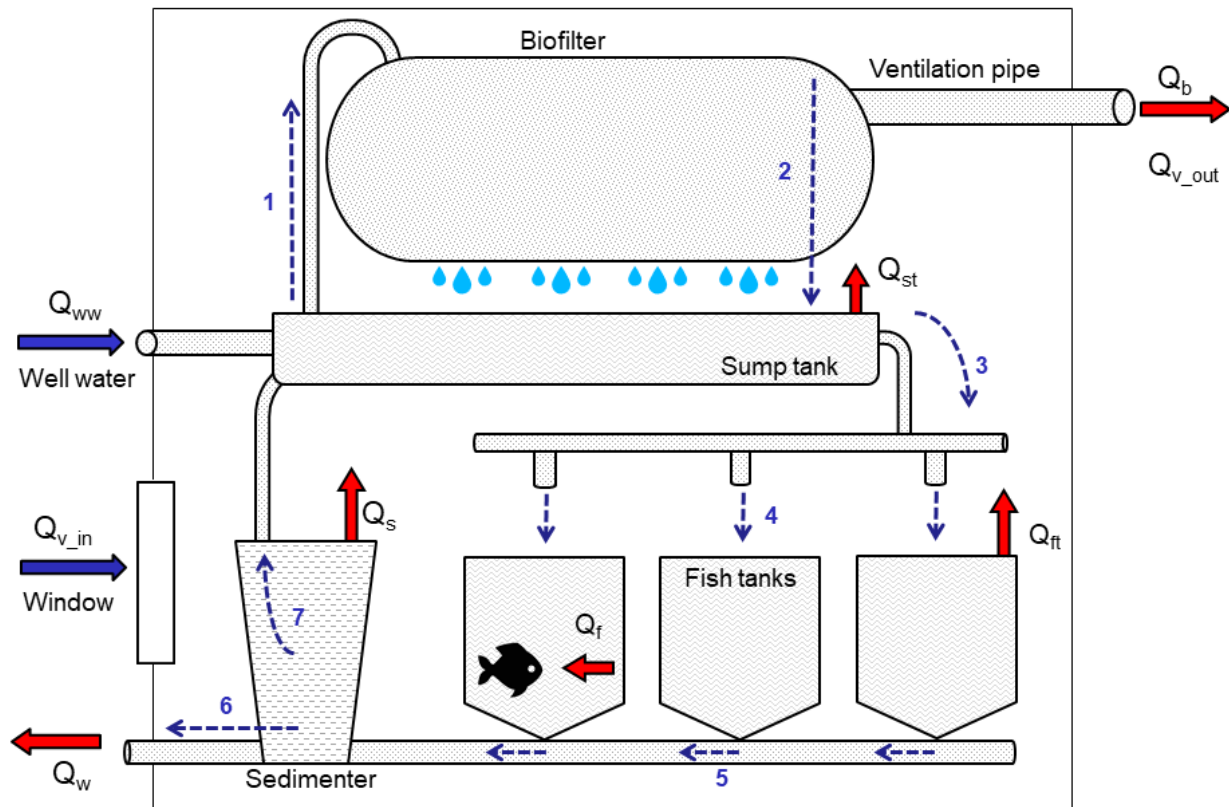
$$\begin{aligned}
 173 \quad Q_b \left[\frac{\text{kg H}_2\text{O}}{\text{s}} \right] &= Q_{b,\text{on}} \left[\frac{\text{kg H}_2\text{O}}{\text{s}} \right] - Q_{b,\text{off}} \left[\frac{\text{kg H}_2\text{O}}{\text{s}} \right] = \\
 174 \quad &\left(\text{AH} \left[\frac{\text{kg}}{\text{m}^3} \right] \cdot v \left[\frac{\text{m}}{\text{s}} \right] \cdot A_w [\text{m}^2] \right)_{\text{on}} - \left(\text{AH} \left[\frac{\text{kg}}{\text{m}^3} \right] \cdot v \left[\frac{\text{m}}{\text{s}} \right] \cdot A_w [\text{m}^2] \right)_{\text{off}} \quad [\text{Eq. 6}]
 \end{aligned}$$

175

176 *Fish water assimilation.* The fish water assimilation (L day^{-1}) was calculated by assuming water being 78.11%
 177 of the final fresh body weight of European catfish (Żmijewski et al., 2006). The final amount of water stored
 178 in the fish biomass was divided by the days of the growing cycle (365).

179

180 *Wastewater.* The amount of discarded water equals the capacity of the sedimenter, which is emptied daily.



181

182 **Figure 1.** Graphical representation of the water flows across the aquaculture system. Dashed lines indicate
 183 the water flow: the well water enters in the sump tank and is raised to the top of the biofilter (1), falls
 184 through the biofilter net (2) and drops down again in the sump tank; from here it is distributed in the three
 185 fish tanks (3 -4); then, flows towards the sedimentor (5) and, once filtered, cycle back to the sump tank (7),
 186 from where restarts its cycle. Once a day, then, the sedimentor is emptied (6). The blue arrows represent
 187 the water input (Q_{ww} Well water consumption, Q_{v_in} Vapour flow entering through the ventilation system).
 188 The red arrows represent the water output (Q_{v_out} Vapour flow exiting through the ventilation system, Q_f
 189 Water assimilated by fishes, Q_{ft} Water evaporated by the fish tanks, Q_s Water evaporated by the sedimentor,
 190 Q_{st} Water evaporated by the sump tank, Q_b Water evaporated from the biofilter, Q_w Water discarded by
 191 the system).

192

193 The RAS efficiency was, finally, evaluated in term of water use ($m^3 H_2O kg^{-1} FW$), by dividing the total annual
 194 water inflow of the system (m^3) by the average annual fish fresh yield (kg) (Verdegem et al., 2006).

195

196 *2.2.2 Evaluation of lettuce cultivation in aquaponics vs traditional hydroponics*

Eight boxes of 30 L were employed to grow up the plants, covered by a polystyrene sheet with ten holes to support the seedlings (transplanted at sixth leaf stage). Four water treatments were imposed at different electrical conductivity (EC): Aqua (water from the RAS with EC 1.6 dS m⁻¹), Hydro 1.6 (hydroponic solution with EC 1.6 dS m⁻¹), Hydro 2.0 (hydroponic solution with EC 2.0 dS m⁻¹) and Hydro 3.0 (hydroponic solution with EC 3.0 dS m⁻¹). The initial composition, pH, EC and C:N ratio of the four nutrient solutions are described in Table 1. All the boxes were connected to an air pump to guarantee oxygenation to the nutrient solution. Air humidity and temperature were recorded by a sensor every ten minutes.

Table 1: Composition of the nutrient solutions of the treatments Aqua, Hydro 1.6, 2.0 and 3.0, respectively.

		Aqua	Hydro 1.6	Hydro 2.0	Hydro 3.0
pH		7.3	6.9	6.7	6.5
EC	dS·m ⁻¹	1.6	1.6	2	3
NH₄⁺	mmol L ⁻¹	0.09	0.27	0.33	0.5
NO₃⁻	mmol L ⁻¹	7.36	6.94	8.73	13.24
K⁺	mmol L ⁻¹	0.58	6.13	7.67	11.5
Na⁺	mmol L ⁻¹	4.32	0.9	1.1	1.2
Ca²⁺	mmol L ⁻¹	3.4	2.93	3.67	5.5
Mg²⁺	mmol L ⁻¹	0.43	0.8	1	1.5
Cl⁻	mmol L ⁻¹	2.77	1.07	1.33	2
S	mmol L ⁻¹	1.35	1.07	1.33	2
HCO₃⁻	mmol L ⁻¹	0.72	0.08	0.1	0.15
P	mmol L ⁻¹	0.09	1.07	1.33	2
Fe	μmol L ⁻¹	0.28	21.33	26.67	40
Mn	μmol L ⁻¹	0.2	2.67	3.33	5
Zn	μmol L ⁻¹	2.22	2.67	3.33	5

B	$\mu\text{mol L}^{-1}$	29.83	26.67	33.33	50
Cu	$\mu\text{mol L}^{-1}$	0.53	0.53	0.67	1
Mo	$\mu\text{mol L}^{-1}$	<0.1	0.27	0.33	0.5
N tot	mg L^{-1}	105.05	100.93	126.94	192.44
C:N ratio		0.083	0.010	0.009	0.009

207

208 *Water consumptions.* The evapotranspirative losses (ET_p) were measured by reading the water level decrease
209 inside the boxes through a graduated scale.

210

211 *Biomass production.* Once harvested, plants' shoots were immediately separated from roots and weighted in
212 order to obtain the fresh weight (FW). Dry weight (DW) was determined after oven-drying the sample for
213 three days at 105°C . According to Qiansheng et al. (2018) the lettuce root biomass in hydroponics accounts
214 for only about the 10% of the total fresh biomass, therefore it was considered negligible and not taken into
215 account for the calculation of the below-listed parameters. The dry matter percentage (DM) was calculated by
216 the ratio between the lettuce head dry (g) and fresh (g) weight, and expressed as percentage.

217

218 *C:N ratio:* Total leaf N and C content, were measured on dry samples with a LECO CNS2000 elemental
219 analyzer (LECO Corporation, St. Joseph, MI, USA).

220

221 *WUE.* Total water use efficiency ($\text{g FW L}^{-1} \text{H}_2\text{O}$) was calculated as the ratio of the FW (g) of the plants of each
222 box to the relative total water consumption (L) (Fallovio et al., 2009).

223

224 *NUE.* Water samples of the nutrient solution were collected at the beginning of exp. 2 and 3. These were
225 analyzed for main macro and microelements by the Eurofins Agro water test laboratories (Binnenhaven 5 NL
226 - 6709 PD, Wageningen, The Netherlands). The NUE ($\text{g FW mg}^{-1} \text{N}$) was calculated by the ratio between the
227 average fresh weight of the plants of each box (g) and the total elemental nitrogen available at the beginning
228 of the growing cycle in the nutrient solution (mg) (Benincasa et al., 2011).

229

230 2.2.3. *Design for a closed water cycle*

231 With the aim of boosting the resources efficiency of the aquaculture system, a DWC hydroponic unit can be
232 introduced in the already existing RAS to recover its water discharge and recycle it as nutrient solution for
233 lettuce cultivation. In such a system, water requirements are only limited to those needed to compensate
234 evaporative water losses. Plants in aquaponics play a key role in water quality control notably as concerns N-
235 and P-containing nutrients (Goddek et al., 2015). For a proper operation of an aquaponics system, therefore,
236 the plant cultivation must be dimensioned to the fish stocking density in order to ensure an efficient control of
237 nutrient load.

238 Keeping the size of the actual RAS system, a design for an aquaponic system for lettuce-catfish production is
239 here proposed. The proportion of the DWC beds is calculated through a parameter elaborated by Rakocy et al.
240 (2006) called Feeding Rate Ratio (FRR) (Eq. 7), which express the relation between the fish feed amount
241 introduced in the system and the plant growing area.

242

$$243 \quad \text{FRR} \left[\frac{\text{g}}{\text{m}^2 \text{d}} \right] = \frac{\text{fish feed [g]}}{\text{plant area [m}^2\text{]} \cdot \text{day}} \quad [\text{Eq. 7}]$$

244

245 The optimum FRR for raft hydroponics varies from 60 to 100 g·m⁻²·day⁻¹ (Rakocy et al., 2006). Accordingly,
246 for the purpose of this study, a FRR of 80 g·m⁻²·day⁻¹ for raft hydroponic lettuce production was set. The
247 computations were done assuming that the production should be split and harvested in staggered phases to
248 keep constant the optimal stocking density (Rakocy et al., 2006). A fish productive cycle of 12 months was
249 assumed to allow obtaining adults of 2 kg (with a final stocking density of 100 kg·m⁻³) from fingerlings of 300
250 g (data established according to the performance of previous cycles and literature data, e.g. Żmijewski et al.,
251 2006). The fish production should be staggered to harvest once a year from each tank, with a time step of about
252 17 weeks (the three harvests being distributed along the year). Starting from a plant at the sixth unfolded leaf
253 stage, a productive growing cycle of 4 weeks was considered, setting a plant density of 16 plants m⁻² (Rakocy
254 et al., 2006).

255

256 2.3. Statistical analysis

The experimental data were treated statistically using CoStat software package. A two-way analysis of variance (ANOVA) at 95% confidence was performed to test the influence of the growing cycle and the water source on evapotranspiration losses, biomass production, WUE and NUE. Means were compared using the LSD test at $P \leq 0.05$.

3. Results and discussion

3.1. RAS water balance and efficiency parameters

The results of the water balance on the aquaculture unit showed that about 555 L of water flow through the system every day. A description of the water metabolism elements is reported in Table 2.

Table 2. Daily water input and output within the aquaculture system.

AQUACULTURE SYSTEMS			
INPUT		OUTPUT	
Air absolute humidity ($\text{kg}\cdot\text{day}^{-1}$)	22.9 ± 3.0	Air absolute humidity ($\text{kg}\cdot\text{day}^{-1}$)	52.6 ± 1.41
		Biofilter ($\text{kg}\cdot\text{day}^{-1}$)	17.7 ± 1.52
Well water ($\text{L}\cdot\text{day}^{-1}$)	532	Sump tank ($\text{kg}\cdot\text{day}^{-1}$)	6.7 ± 0.26
		Fish tanks ($\text{kg}\cdot\text{day}^{-1}$)	5.8 ± 0.16
		Sedimenter ($\text{kg}\cdot\text{day}^{-1}$)	0.9 ± 0.09
		Fish water assimilation ($\text{L}\cdot\text{day}^{-1}$)	0.70
		Water discharge ($\text{L}\cdot\text{day}^{-1}$)	460
		Undetermined losses ($\text{L}\cdot\text{day}^{-1}$)	10.45
TOTAL INPUT	554.9 ± 3.0	TOTAL OUTPUT	554.9 ± 3.44

The biofilter produced the highest evaporation losses ($17.7 \text{ L}\cdot\text{day}^{-1}$), which resulted 62.5%, 67.2% and 95.2% higher than those generated from the sump, the fish tanks and the sedimenter (6.7 , 5.8 , and, $0.9 \text{ L}\cdot\text{day}^{-1}$), respectively. This could be due to a peculiarity of the biofilter used in this system which exploits the water evaporation to cool down the air temperature.

Water use for catfish in the experimental RAS was $0.59 \text{ m}^3\cdot\text{kg}^{-1}$, 2.5 folds higher than that recorded for tilapia production in RAS ($0.24 \text{ m}^3\cdot\text{kg}^{-1}$) (Eurofish, 2009). Previous experiences also suggest that water consumption values are highly variable in response to the fish productive system adopted: they range from 0.5 to $0.7 \text{ m}^3 \text{ kg}^{-1}$.

276 ¹ in a super-intensive recirculating system (Verdegem et al., 2006) or average 1 m³·kg⁻¹ in a RAS system
277 (Bregnballe, 2015) but also increase up to 45 m³·kg⁻¹ in an extensive pond system (Verdegem et al., 2006).
278 The average fish yield of the studied RAS was 329 kg·year⁻¹, which corresponds to a total amount of water
279 assimilated by the fish of 257 L.

280

281 **3.2 Evaluation of lettuce cultivation in aquaponics vs traditional hydroponics**

282

283 ***Climatic condition.*** Exp. 1 was performed between July and August 2016. The minimum, average and
284 maximum temperatures were respectively 17.0, 28.4 and 46.0°C in July and 13.7, 23.5 and 34.4°C in August.
285 The mean daily temperature was higher than the average monthly temperature for 13 days. Exp. 2 was
286 performed between the last days of September and October 2016. The minimum, average and maximum
287 temperatures were respectively 14.5, 21.1 and 34.0°C in September and 12.0, 18.0 and 30.5°C in October. The
288 mean daily temperature was higher than the average temperature for 17 days. Exp. 3 was performed between
289 June and July 2017. The minimum, average and maximum temperatures were respectively 16.0, 24.3 and
290 50.0°C in June and 17.0, 23.9 and 39.0°C in July. Mean daily temperature was higher than the average
291 temperature for 20 days. The absence of an automated system for temperature management did not allow to
292 prevent the occurrence of extreme temperatures.

293

294 ***Water and nutrient consumption.*** The water consumptions did not show statistically significant differences
295 (P-value>0.05) among the four treatments (Hydro 1.6, Hydro 2.0, Hydro 3.0 and Aqua), although seasonal
296 variations in total water consumption were observed among the three experiments. The average daily amount
297 of evapotranspiration was 52.0, 27.3 and 58.4 mL·head⁻¹·day⁻¹ for the exp. 1, 2 and 3, respectively. Similar
298 results were observed by Ciolkosz et al. (1998) and Conversa et al. (2004), who recorded respectively ET
299 values comprised between 24 and 178 mL·head⁻¹·day⁻¹ and between 76 to 214 mL·head⁻¹·day⁻¹ in lettuce grown
300 in hydroponics. The lowest ET value were recorded in the exp. 2, which coincided with the lower temperatures
301 experienced during the autumn season. The composition of the nutrient solutions at the end of the experiments
302 is reported in Table 3. In all cycles the EC of the treatments Hydro 1.6 and 2.0 was reduced from the initial
303 value, whereas an increase in EC in both Aqua and Hydro 3.0 treatments was observed during cycles 1 and 3.

304 A pH increase was observed for all the nutrient solutions in every cycle, with special emphasis for the
 305 treatments Hydro 1.6 and 2.0. The ammonium concentration dropped close to zero in all the treatments, while
 306 the nitrate concentration increased, especially in hydroponic. The potassium, phosphorus, molybdenum and
 307 manganese concentrations in aquaponics, already low at the beginning of the experiment, reached almost zero
 308 at the end of the three cycles, while the final potassium and molybdenum concentration in hydroponics was
 309 increased from the initial value, possibly as a consequence of water evaporation and root selective uptake
 310 (Albornoz and Lieth, 2015). The aquaponics iron concentration, instead, remained almost constant or
 311 increased, probably due to both the solution evaporation and the lower iron availability when pH is above 7.
 312 Calcium concentration, as well, did not vary largely in aquaponics while it was one forth on average in the
 313 other solutions. This might be attributed to the uptake competition played by the sodium ion, since its initial
 314 concentration in aquaponics was 4-folds higher than in hydroponics (Albornoz and Lieth, 2015).

315

316 **Table 3:** Composition of the nutrient solutions of the treatments Aqua, Hydro 1.6, 2.0 and 3.0 at the end of the
 317 three experiments.

		Aqua			Hydro 1.6			Hydro 2.0			Hydro 3.0		
Cycle		1	2	3	1	2	3	1	2	3	1	2	3
pH		7,52	7,76	7,65	7,55	7,9	7,7	6,9	7,14	7,7	6,4	7,05	6,95
EC	dS·m ⁻¹	1,85	1,3	1,8	1,5	1,2	1,25	1,8	1,45	1,25	3,25	2,65	3,05
NH₄⁺	mmol L ⁻¹	<0,1	<0,1	< 0,1	<0,1	<0,1	< 0,1	<0,1	<0,1	< 0,1	<0,1	<0,1	< 0,1
NO₃⁻	mmol L ⁻¹	9,2	5,7	4,85	1,8	0,4	< 0,1	3,45	1,6	< 0,1	8,7	6,3	4,25
K⁺	mmol L ⁻¹	0	<0,1	< 0,2	9	6,45	8,4	11,9	8,35	8,4	24,1	17,6	22,6
Na⁺	mmol L ⁻¹	5,35	3,55	9,65	1,4	1,4	1,95	1,2	1,3	1,95	1,35	1,55	2,2
Ca²⁺	mmol L ⁻¹	4,8	3,5	4,5	0,65	0,65	0,75	0,85	0,85	0,75	2	1,4	1,1
Mg²⁺	mmol L ⁻¹	0,55	0,4	0,5	0,25	0,2	0,2	0,2	0,2	0,2	0,3	0,3	0,2
Cl⁻	mmol L ⁻¹	3,85	2,55	5,05	2,75	2,55	2,25	3,05	2,8	2,25	5,1	4,75	5,65
S	mmol L ⁻¹	2,05	1,5	2,55	2,9	2,35	3,8	3,25	2,75	3,8	5,35	4,35	6,35
HCO₃⁻	mmol L ⁻¹	0,15	0,8	3,85	0,4	1,5	0,95	0,1	0,9	0,95	<0,1	0,35	0,2
P	mmol L ⁻¹	<0,04	<0,04	< 0,04	0,575	0,42	0,81	1,35	0,59	0,81	3,055	1,325	2,32
Fe	μmol L ⁻¹	0,35	0,2	0,55	21,5	12	28,5	11,15	16,5	28,5	17,5	23	81,5
Mn	μmol L ⁻¹	0,1	< 0,1	< 0,1	0,2	0,15	0,15	0,1	0,15	0,15	1,75	0,8	0,25
Zn	μmol L ⁻¹	2,4	2,85	0,15	24	7,4	8,35	48,5	8,65	8,35	75	30,5	14

B	$\mu\text{mol L}^{-1}$	36	21,5	91	53	44	82	65,5	58	82	110	99,5	143
Cu	$\mu\text{mol L}^{-1}$	0,35	0,45	0,95	1,3	1,4	3,15	1,1	1,7	3,15	2,05	1,7	3,85
Mo	$\mu\text{mol L}^{-1}$	0	< 0,1	< 0,1	1,7	0,4	0,85	1,35	0,4	0,85	1,55	0,6	1,3
N tot	mg L^{-1}	130,12	81,09	69,19	26,47	6,86	2,52	49,58	23,67	2,52	123,12	89,50	60,79
C:N ratio		0,014	0,118	0,668	0,181	2,625	4,526	0,024	0,457	4,526	0,009	0,047	0,040

318

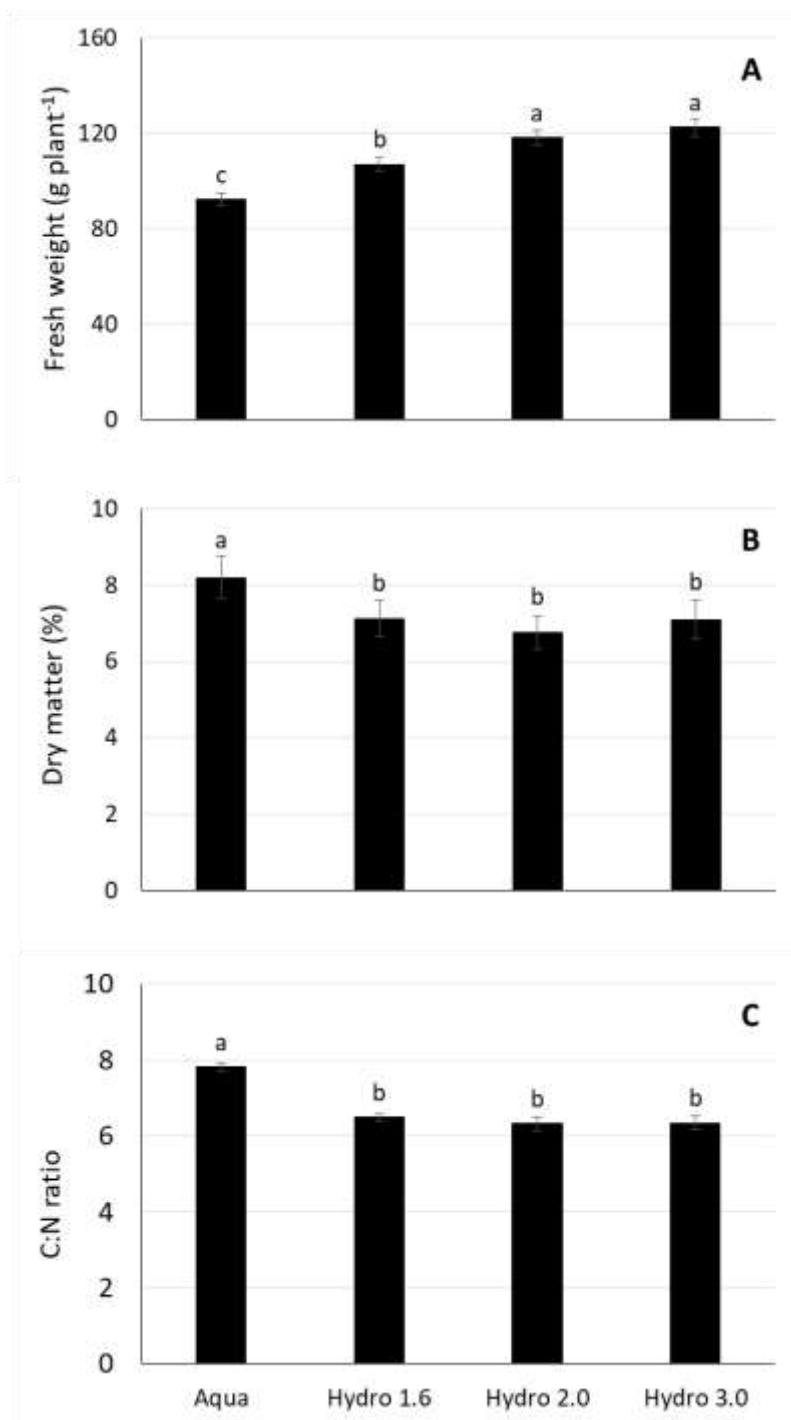
319 **Yield.** Considering that not statistically significant interactions were detected between the two experimental
320 factors (growing cycle and water source), mean values are used for presenting yield data. Lettuce grew the
321 least in aquaponics ($92.3 \text{ g}\cdot\text{plant}^{-1}$), whereas higher biomass productions were achieved in the Hydro 2.0 and
322 3.0 (mean value of $120.3 \text{ g}\cdot\text{plant}^{-1}$) similarly to previous studies on aeroponically grown lettuce (Albornoz and
323 Lieth, 2015). The absence of yield differences between Hydro 2.0 and Hydro 3.0 shows that nutrients in the
324 latter resulted in luxury consumption rather than increasing biomass production nor leading to salinity
325 symptoms (Nozzi et al., 2018).

326 A lower performance in aquaponics versus hydroponics was previously described by El Sayed and Samir
327 (2015), and Johnson et al. (2017), whereas Pantanella et al. (2012) and Delaide et al. (2016) did not observe
328 differences in yield among the two growing systems. The lower yield in aquaponics may be associated to
329 reduced K^+ , P^+ , Fe^{2+} and Mn^{2+} concentrations in the nutrient solution (Rakocy et al., 2007). The amount of
330 these nutrients in aquaponics is often not adequate to the plant requirements due to their low concentration in
331 most of the commercial fish feeding formulations, and because part of them precipitate and is lost in the form
332 of fish solid excretion (Rakocy et al., 2007; Goddek et al., 2015). Besides, to counteract the pH drops due to
333 the bacterial nitrification process, a bicarbonate buffer was periodically added to the RAS circulating medium.
334 The subsequent higher HCO_3^- concentration in the aquaponics solution, combined with the higher pH (Table
335 1), may have contributed to reduce the nutrient solubility and absorbability (Pignata et al., 2017), especially
336 for phosphorous, iron, manganese, magnesium and calcium (Trejo-Téllez and Gómez-Merino, 2012).
337 Furthermore, it has also been suggested that the bacterial community from the aquaponics may compromise
338 the nutrient availability by consuming nutrients or by increasing the energetic cost to import them across the
339 root interface (Wielgosz et al., 2017). Goddek et al. (2015) stated that aquaponics can match hydroponics when
340 all the parameters are controlled, and the bacterial community is fully mature. In the current study, the nutrient

341 imbalance in the aquaponics water solution may indicate that the fish stocking rate and/or their dietary
342 composition need to be adjusted to supply the plants with the proper nutrients amount.

343 Despite the lowest yield, however, the DM% and C:N ratio in leaf tissues were significantly higher in
344 aquaponics than in hydroponics (Fig. 1). This may depend on the different nutrient composition and availability
345 in the two growing systems. As already mentioned, indeed, the C:N ratio of the nutrient solution in aquaponics
346 was, 8.7-fold higher than the average value in the three hydroponic treatments (Table 1). Moreover, as stated
347 by Nozzi et al. (2018) a P deficiency in the nutrient solution (Table 1) reduces the root N uptake. This, in turn,
348 induces accumulation of non-structural carbohydrates (e.g. organic acids and sugars) for the cellular
349 osmoregulation, resulting in a DM increase and, consequently, also in changes in the C:N ratio. The absence
350 of significant variations in DM among the hydroponic treatments, on the other hand, is in line with the result
351 of Ünlükara et al. (2008) and Scuderi et al. (2009) which reported a nearly constant DM content in plants
352 growing under variable salinities, even over 3 dSm⁻¹.

353



354

355 **Figure 1.** Fresh weight (A), dry matter (B), and C:N ratio (C) of lettuce plants grown under different water
 356 regimes. Data referring to exp. 1, 2 and 3 are indicated as mean \pm SE. Different letters indicate significant
 357 differences at $P \leq 0.05$.

358

359 **NUE.** Given that the interaction between the two experimental factors (growing cycle and water source) was
 360 not statistically significant, the mean values for NUE were used. In the hereby presented experiments, NUE in
 361 aquaponics ($0.34 \text{ g FW} \cdot \text{mg}^{-1} \text{ N}$) was significantly lower than in Hydro 1.6 and 2.0 ($0.5 \text{ g FW} \cdot \text{mg}^{-1} \text{ N}$ on

average), but statistically non different from the Hydro 3.0 treatment ($0.33 \text{ g FW} \cdot \text{mg}^{-1} \text{ N}$). The lower performance in aquaponics may, again, depend on the different ratio of nitrogen forms in the nutrient solution. Ammonia ($\text{NH}_4\text{-N}$) level in aquaponics was, in fact, below $0.1 \text{ mmol} \cdot \text{L}^{-1}$ (Table 1) due to the nitrification operated by the bacteria of the RAS biofilter. Despite most plants prefer NO_3^- over NH_4^+ as nitrogen source, the NO_3^- acquisition and assimilation is more energy demanding than ammonium and, then, a 1:3 ratio of $\text{NH}_4^+:\text{NO}_3^-$, compared with nitrate alone as the sole source of N, showed to be beneficial to plant growth and yield in hydroponics (Savvas et al., 2006).

Increasing the nitrogen fertilization and uptake may not necessarily lead to improved crop yield and NUE (M'hamdi et al., 2014). A similar behaviour was observed in the present experiment when, rising the EC up to $3 \text{ dS} \cdot \text{m}^{-1}$, (N concentration of $243 \text{ mg} \cdot \text{L}^{-1}$), the yield did not increase, leading to a decrease in NUE (Fig. 2). This is consistent with the findings of Stefanelli, Winkler and Jones (2011) who reported increased lettuce NUE at N concentration from 40 to $75 \text{ mg} \cdot \text{L}^{-1}$, reaching a plateau at $150 \text{ mg} \cdot \text{L}^{-1}$ and with no subsequent increases between 400 and $2400 \text{ mg} \cdot \text{L}^{-1} \text{ N}$. Similarly, according to Mahlangu et al. (2016), a N concentration of $100\text{-}120 \text{ mg} \cdot \text{L}^{-1}$ is enough to improve growth, yield and quality parameters of hydroponic lettuce. In the present case, the N concentration at $2.0 \text{ dS} \cdot \text{m}^{-1}$ ($162.0 \text{ mg} \cdot \text{L}^{-1} \text{ N}$) enhanced NUE and maximized the production, while the increased nitrogen input in Hydro 3.0 ($243 \text{ mg} \cdot \text{L}^{-1} \text{ N}$) resulted in a NUE decline with no further yield increase.

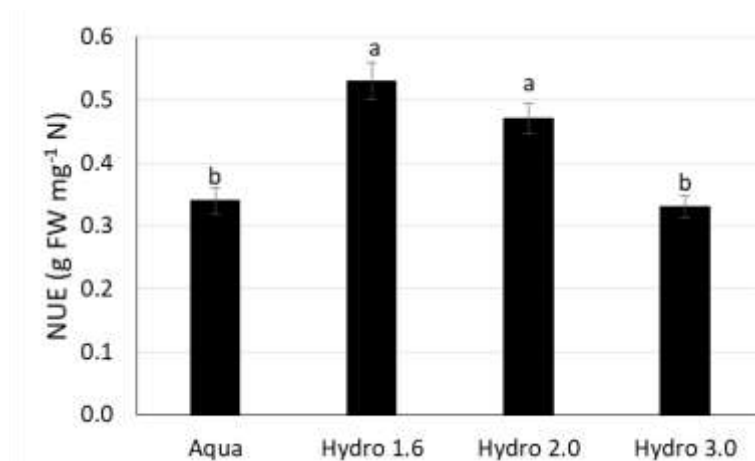


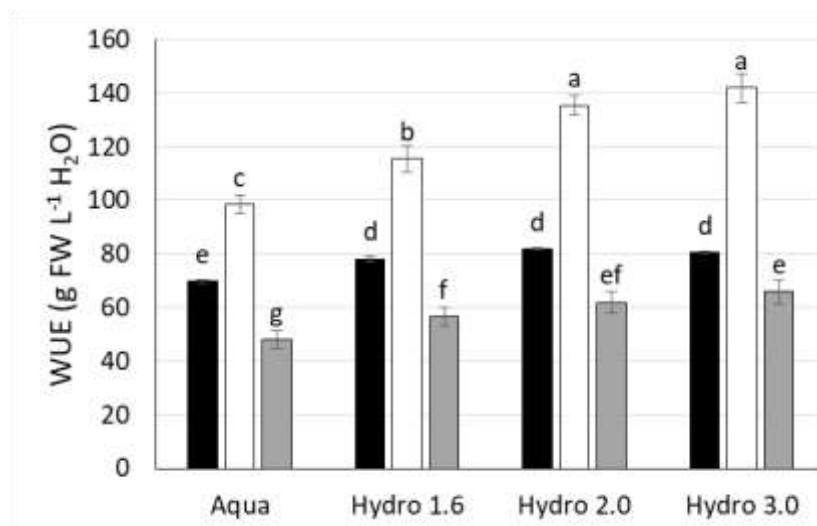
Figure 2. Nitrogen use efficiency (NUE) of lettuce plants grown under different water regimes. Data referring to exp. 2 and 3 are indicated as mean \pm SE. Different letters indicate significant differences at $P \leq 0.05$.

WUE. The interaction between the growing season and the water source significantly affected the lettuce WUE. Aquaponics always showed the lowest WUE [69.8 g FW·L⁻¹ H₂O for exp. 1, 98.5 g FW·L⁻¹ H₂O for exp. 2 and 48.1 g FW·L⁻¹ H₂O for exp. 3] (Fig. 3). This is probably due to a decrease in the photosynthetic efficiency rather than in the leaf evaporation as no difference in water consumption arose among aquaponics and hydroponics treatments (De Costa and Ariyawansa, 1996). In addition, it is worth to mention that Na⁺ concentration in aquaponics was 4-folds higher than in hydroponics (Table 1). As already observed by Tzortzakis (2009), moderate sodium stress is more likely to affect elemental absorption by competition than interfering with the root water uptake, leading to ions imbalance, restricted nutrient uptake and consequent yield reduction. The production of osmolytes to counteract the specific Na⁺-related osmotic stress may be another justification of the higher leaf DM encountered in aquaponics. In conclusion, the nutrient imbalance in aquaponics, namely the higher sodium concentration combined with the lack in potassium, phosphorous, iron and manganese, had a more negative impact on plant yield and WUE than the higher water conductivity in the hydroponic treatments.

In hydroponics, different WUE trends were observed in the three cycles. In exp. 1 no WUE differences arose between the three treatments. In exp. 2, the WUE of the Hydro 2.0 and 3.0 treatments were comparable but resulted significantly higher than the Hydro 1.6. In exp. 3, significant differences could only be found between Hydro 1.6 and Hydro 3.0, the latter presenting higher values. According to the obtained results, it may be argued that an EC increase over 1.6 dS·m⁻¹ could possibly boost WUE. Moreover, although significant differences could be observed between the treatments Hydro 1.6 and Hydro 3.0, the latter never differed significantly from the intermediate Hydro 2.0. Hence, bearing in mind the objective to contain the productive costs and the chemical input, using an EC of 2.0 in place of 1.6 dS·m⁻¹ can boost the crop yield without interfering with the NUE and WUE. The lower WUE observed in the summer cycles (exp. 1 and 3) may be due to the higher solar radiation and temperature which may have increased the atmospheric evaporative demand and the plant respiratory losses (Falovo et al., 2009) as compared with the autumn cycle (exp. 2). A similar behaviour was observed also by Shaban et al. (2016), who observed a WUE increase when lettuce was subjected to irrigation water cooling and shading. Lettuce, indeed, is a short-day cool season crop with an optimum temperature range of 7-24 °C (Shaban et al., 2016). Under warmer condition the plant increase the transpiration rate to regulate the leaf temperature, through the mechanism of

412 evaporative cooling. However, although transpiration is positively correlated to biomass accumulation, upon
 413 elevate temperature the plant reduces its ability to regulate the water relations and further transpiration losses
 414 do not provide extra biomass gain, lowering thereby the WUE (Zhang et al., 2015). The high temperature,
 415 combined with the high radiation, may have also resulted in reaching the light saturation point, inducing
 416 photoinhibition and stomatal closure (Hunt et al., 1984). Hence, it may be argued that the decreased
 417 photosynthesis efficiency and carbon assimilation, associated with an increase in the canopy transpiration,
 418 might have negatively affected the plant WUE during the two summer cycles.
 419 The achieved WUE values are, however, consistent with the results of Chabite et al. (2017) that also
 420 obtained values among 29.7 and 142.9 g·L⁻¹ for lettuce grown using different nutrient solutions.

421



422

423 **Figure 3.** Water Use Efficiency (WUE) of lettuce plants grown under different water regimes. Black bars
 424 represent results of exp. 1, white bar represent results of exp. 2, grey bar represent results of exp. 3. Data are
 425 expressed as mean ± SE. Different letters indicate significant differences at $P \leq 0.05$.

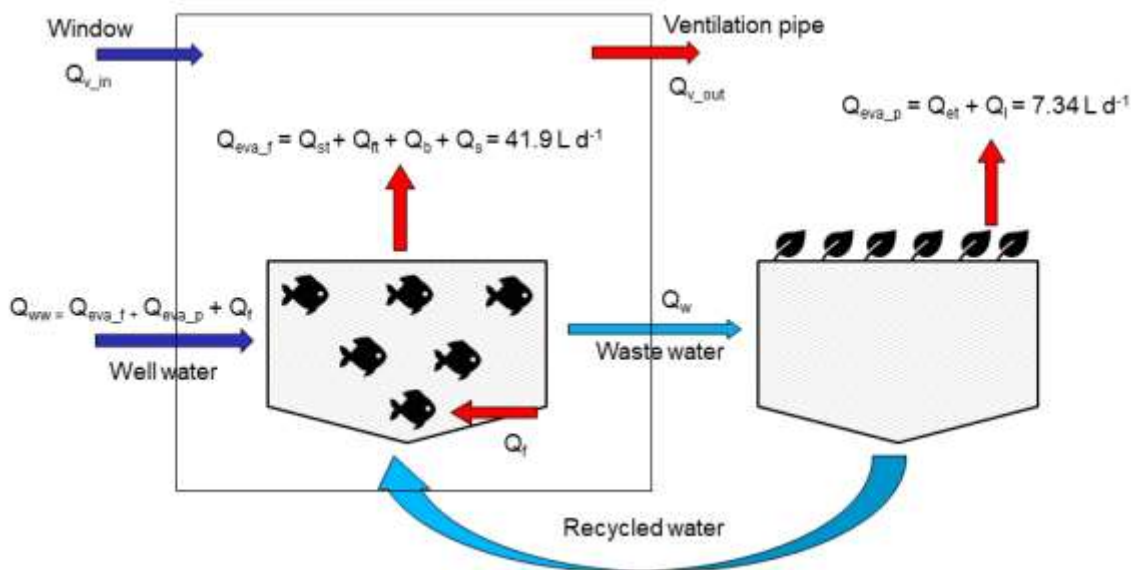
426

427 3.2. Design proposal to convert the RAS into a catfish-lettuce aquaponic system

428 In this section, a design for introducing a lettuce DWC hydroponic unit into the already existing RAS is
 429 proposed, with the goal to transform it in a closed productive aquaponic system.

430 According to Eq. 1 and the data from the Table 2, the total water input and output within the RAS consisted
 431 of 554.9 L·day⁻¹. The estimated potential catfish production of the current RAS is 329 kg·year⁻¹. According to

432 the calculation procedure elaborated by Rakocy et al. (2006), a lettuce growth bed area of 9.63 m^2 is required
 433 to satisfy the nitrogen filtration demand at this productive rate. Keeping the suggested plant density of 16 plants
 434 m^{-2} , the projected 10 m^2 DWC plant may host up to 160 lettuces per month. According to the attained results
 435 on the lettuce mean water consumption, i.e. $45.9 \text{ mL} \cdot \text{head}^{-1} \cdot \text{day}^{-1}$, an average water consumption (Q_{eva_p}) of
 436 $7.3 \text{ L} \cdot \text{day}^{-1}$ is expected for a raft system hosting 160 plants. The current well water input ($Q_{\text{ww}} = 532 \text{ L} \cdot \text{day}^{-1}$)
 437 could, therefore, be reduced to the amount needed to replace the water lost by evaporation from the RAS (Q_{eva_f}
 438 $= 41.6 \text{ L} \cdot \text{day}^{-1}$), from the hydroponics tubs ($Q_{\text{eva}_p} = 7.3 \text{ L} \cdot \text{day}^{-1}$), and the summed water withheld in the fish
 439 tissues ($Q_f = 0.7 \text{ L} \cdot \text{day}^{-1}$) (Figure 4). With the introduction of the proposed hydroponic unit, then, the RAS
 440 water discharge ($Q_w = 460 \text{ L} \cdot \text{day}^{-1} \cdot \text{m}^{-2}$) will be fetched to supply the DWC system and the system water
 441 metabolism will improve as the water output will decrease from 554.9 to $102.53 \text{ L} \cdot \text{day}^{-1}$ (Table 4).
 442 By installing an environment control system in the greenhouse, the lettuce production can be realized
 443 continuously over the 365-days cycle of catfish. A staggered crop production system, however, is suggested
 444 to keep the water quality relatively constant and allow the lettuce harvest with regular cadency. Assuming a
 445 30-day crop cycle, then, the lettuce production can be staggered so that four growth stages can be
 446 simultaneously cultivated in one month and one-fourth of the lettuces can be weekly harvested (40 lettuce
 447 heads). For the fish production, as well, three fish ages should be contemporary reared in the three tanks in
 448 order to produce an effluent whose composition remains relatively constant. Accordingly, the fish from one
 449 tank will be stocked once every four months, with an expected potential production of $82.3 \text{ kg quadrimester}^{-1}$.
 450 The yearly production, then, will consist of 329 kg of fish meat plus 1920 lettuce heads, which correspond to
 451 a biomass production of $17.7 \text{ kg} \cdot \text{m}^{-2}$, if the average lettuce FW obtained in aquaponics during the hereby study
 452 ($92.3 \text{ g} \cdot \text{plant}^{-1}$) is considered.
 453



454

455 **Figure 4.** Graphical representation of the water flows across the proposed catfish-lettuce aquaponic system.

456 The dark-blue arrows represent the water input: Q_{ww} Well water consumption, Q_{v_in} Vapour flow entering
 457 through the ventilation system. The red arrows represent the water output: $Q_{eva_p} = (Q_{ft}$ Water evaporated
 458 by the fish tanks + Q_s Water evaporated by the sedimenter + Q_{st} Water evaporated by the sump tank + Q_b
 459 Water evaporated from the biofilter), Q_f Water assimilated by fishes, Q_{v_out} Vapour flow exiting through
 460 the ventilation system, Q_w Water discarded by the system, $Q_{eva_p} = (Q_{et}$ Water evaporated by the raft
 461 hydroponic system + Q_l Water assimilated by the plant tissue. The light-blue arrows represent the circular
 462 water pathway among the two aquaculture and hydroponic sub-units.

463

464 **Table 4.** Comparison of the water input-output and of the marketable products obtainable from the current
 465 RAS system and from the proposed catfish-lettuce aquaponics system. All the water-related items are
 466 expressed in $L\ day^{-1}$: Q_{ww} Well water consumption, Q_{v_in} Vapour flow entering through the ventilation
 467 system, Q_{eva} Water evaporated by the aquaculture tanks and/or the hydroponic tanks, Q_f Water assimilated
 468 by fishes, Q_{v_out} Vapour flow exiting through the ventilation system, and Q_w Water discarded by the system.

Total input	water input	Well input	water output	Total water output	Water discharge	Evaporation losses	Yield
$(Q_{v_in} + Q_{ww})$		(Q_{ww})		$(Q_w + Q_{eva} + Q_f + Q_{v_out})$	(Q_w)	(Q_{eva})	

Current scenario	554.9	532	554.9	460	41.6	360 kg year ⁻¹ fish meat
Proposed scenario	72.84	49.64	102.53	/	48.94	360 kg year ⁻¹ fish meat 1920 lettuce heads year ⁻¹

469 4. Conclusion

470 The present study contributes to the research in aquaponics, offering innovative figures on the water
471 consumption of a catfish recirculating aquaculture and the potential for wastewater saving associated with
472 lettuce production in aquaponics. The results of this study indicated that the considered RAS aquaculture
473 system has a daily water consumption of 555 L·day⁻¹, out of which the 83% is direct water discharge from the
474 system while evaporation losses from the system' tubs account for 31 L·day⁻¹ and humidity detracton from
475 the plant room accounts for 53 L·day⁻¹. With the introduction of a hydroponic component into the system, the
476 discharged water can be recovered and used as nutrient solution for a 10 m² raft hydroponic system. Additional
477 researches are, however, needed to lower the component of evaporative losses by the system.

478 The nutrients requiring supplementation in the hydroponic unit would be ammounium, potassium,
479 phosphorous, iron and manganese, whereas the amount of dissolved sodium and bicarbonate ions may be too
480 elevated to sustain plant productivity. Such nutrient imbalance may be the reason of the lower observed yield,
481 WUE and NUE in aquaponics. Given the already high level of nitrate and sodium, a further increase in the fish
482 stocking density should be avoided while the strategy of integrating the aquaponic solution with synthetic
483 mineral elements, as proposed by Delaide et al. (2016), could be tested to improve the nutrient solution
484 composition. This option would still allow reduced fertilizer costs and environmental impacts compared to
485 traditional hydroponic, but more in-depth investigations would be necessary to determine the nutrient
486 supplementation effects on fish physiology. A change in the fish feeding diets should also be considered. Fish
487 feed contains 0.1 to 0.3% added sodium (Mallick and Rahman, 2005), although such high concentrations is
488 not really necessary to fishes (Rakocy et al., 2007). In order to reduce the levels of Na⁺ in the fishery
489 wastewater, then, alternative feed formulations having higher level of potassium and plant-protein, instead of
490 animal-protein, should be tested for combined catfish-lettuce growth. Finally, studies on the role played by

491 both microorganism and organic compounds on the crop performances could contribute to make this picture
492 more complete.

493 In conclusion, the aquaponics system overall environmental sustainability builds on the avoided impact
494 associated with both the RAS wastewater released into the environment and the less chemical input needed for
495 lettuce hydroponic production as compared with two systems operating independently.

496

497 **Acknowledgements**

498 The authors would like to thank Prof. Lorenzo Barbanti for critically revising the manuscript.

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